JCM
Journals.ASM.org

Optimal Culture Incubation Time in Orthopedic Device-Associated Infections: a Retrospective Analysis of Prolonged 14-Day Incubation

Nora Schwotzer,[a]* Peter Wahl,[b] Dominique Fracheboud,[c] Emanuel Gautier,[b] Christian Chuard[a,d]

Department of Internal Medicine,[a] Department of Orthopedic Surgery and Traumatology,[b] Microbiology Laboratory,[c] and Division of Infectious Diseases,[d] Hôpital Cantonal de Fribourg, Fribourg, Switzerland

Accurate diagnosis of orthopedic device-associated infections can be challenging. Culture of tissue biopsy specimens is often considered the gold standard; however, there is currently no consensus on the ideal incubation time for specimens. The aim of our study was to assess the yield of a 14-day incubation protocol for tissue biopsy specimens from revision surgery (joint replacements and internal fixation devices) in a general orthopedic and trauma surgery setting. Medical records were reviewed retrospectively in order to identify cases of infection according to predefined diagnostic criteria. From August 2009 to March 2012, 499 tissue biopsy specimens were sampled from 117 cases. In 70 cases (59.8%), at least one sample showed microbiological growth. Among them, 58 cases (82.9%) were considered infections and 12 cases (17.1%) were classified as contaminations. The median time to positivity in the cases of infection was 1 day (range, 1 to 10 days), compared to 6 days (range, 1 to 11 days) in the cases of contamination ($P < 0.001$). Fifty-six (96.6%) of the infection cases were diagnosed within 7 days of incubation. In conclusion, the results of our study show that the incubation of tissue biopsy specimens beyond 7 days is not productive in a general orthopedic and trauma surgery setting. Prolonged 14-day incubation might be of interest in particular situations, however, in which the prevalence of slow-growing microorganisms and anaerobes is higher.

Surgical implants play a major role in orthopedic trauma surgery and in the management of degenerative and inflammatory joint diseases. However, the rising number of indwelling devices is associated with increases in related complications. Along with device loosening or malfunctions and foreign-material reactions, infection remains one of the most serious problems encountered with surgical implants. Although orthopedic device-associated infections (ODAI) are uncommon, occurring in only 1 to 2% of patients with hip and knee replacements and up to 6% of patients after internal fixation of closed fractures, their management is difficult (1). Management can require multiple revision surgeries and prolonged antibiotic treatment, may result in permanent disabilities, and is associated with high costs (2, 3).

Despite the promising results reported with newer techniques, such as sonication cultures and molecular testing, the diagnosis of ODAI remains a medical challenge, as routinely used methods lack sensitivity and specificity (4–7). Synovial fluid sample culture, tissue biopsy specimen culture, and histopathological examination show high sensitivities and are frequently considered the gold standard. A reliable microbiological diagnosis is crucial for determining appropriate treatment (8).

There is currently no consensus regarding the appropriate incubation time for ODAI tissue biopsy specimens. The duration of incubation is not specified in most studies, but a 5-day period has often been reported (9–11). Recently, some authors have proposed prolonging the incubation period to 7 or 14 days in order to reveal microorganisms with low virulence, such as *Propionibacterium acnes*, *Peptostreptococcus* spp., and *Corynebacterium* spp. (12–15). Low-virulence, foreign-material-adherent bacteria are typically in a dormant starved state with a slow replicating rate (16). This particular behavior may require a longer culture incubation time (16–19). However, prolonging the incubation time is costly and labor-intensive and could increase the likelihood of detecting organisms that are not clinically relevant. Thus, the aim of our study was to determine if an incubation time of 14 days for tissue biopsy specimens is useful in the diagnosis of ODAI.

MATERIALS AND METHODS

Study design. Microbiological samples of tissue biopsy specimens that were taken from orthopedic device revision surgery (joint replacement and internal fixation devices) between August 2009 and March 2012 and were incubated for 14 days were analyzed. At our institution, 14-day incubation is standard for implant-associated samples and is performed on request for other bone and joint infections. In this study, case identification was prospective and continuous, while the study was retrospective. When there were several interventions for the same joint, only the first revision surgery was considered. The time until microbial growth was recorded. In cases of polymicrobial growth, infection was diagnosed if at least one microorganism fulfilled the diagnostic criteria (see below). The day of growth of the most slowly growing microorganism was used to avoid overlooking late-growing bacteria.

The study was performed in a hospital acting as a primary care and referral center for a population of about 280,000 inhabitants. Elective orthopedic surgery and trauma surgery each account for about one-half of the activity of the Department of Orthopedic Surgery and Traumatology at this hospital. Medical records were reviewed in order to determine if infection was present. Infection was diagnosed according to predefined

Received 19 July 2013 Returned for modification 15 August 2013
Accepted 12 October 2013
Published ahead of print 23 October 2013
Editor: R. Patel
Address correspondence to Nora Schwotzer, nora.schwotzer@chuv.ch, or Christian Chuard, christian.chuard@h-fr.ch.
* Present address: Nora Schwotzer, Department of Internal Medicine, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland.

doi:10.1128/JCM.01766-13

diagnostic criteria (see below). Cases were reviewed by an infectious disease specialist and an orthopedic surgeon. Patient files were scanned for indications of clinical signs of infection (fever, erythema, edema, local hyperthermia, wound discharge, and/or the presence of a sinus tract). A temperature above 38.5°C was considered fever, and fracture nonunion was taken as a potential sign of infection. Preoperative antimicrobial treatment was defined as the administration of any type of antibiotic for more than 24 h during the 14 days preceding surgery. The histopathological findings were divided into 3 categories depending on the average number of polymorphonuclear cells (PMN) per high-power field (HPF) (×400 magnification) on microscopic analysis, as a mean value of at least 10 fields examined, i.e., <1 PMN/HPF, 1 to 5 PMN/HPF, or >5 PMN/HPF.

Definition of infection. Infection was diagnosed if one of the following criteria was fulfilled: (i) positive culture with ≥3 positive samples showing identical microorganisms (20) (microbiological criterion), (ii) positive culture with any number of positive samples and histopathological examination showing >5 PMN/HPF not explained by an acute fracture (21–23) (histopathological criterion), or (iii) positive culture with any number of positive samples and clinical signs of infection, i.e., erythema, edema, local hyperthermia, wound discharge, presence of a sinus tract, or fracture nonunion (8, 24) (clinical criterion). Patients who had not been treated postoperatively with antibiotics and who showed no signs of infection after 12 months of follow-up were not considered to be infected, independent of the diagnostic criteria. Cases with positive cultures that did not fulfill the criteria for infection were classified as contamination.

Culture methods. Tissue sampling was performed in the operating room according to usual surgical methods. The standard procedure was to obtain 3 to 6 samples, with priority given to tissue biopsy specimens if not limited by anatomical restrictions (as in the finger, hand, and foot) (20, 25–27). In order, tissues were sampled from the inflammatory membrane around the implant, the joint capsule, and any macroscopically suspect tissue (28, 29). Each biopsy specimen was stored in transportation medium (BBCPort-A-Cul; Becton, Dickinson, and Company, Sparks, MD) to ensure the survival of all bacteria, including anaerobic microorganisms.

Homogenization of the tissue biopsy specimens was carried out using a disposable closed tissue homogenization system (gentleMACS dissociator; Miltenyi Biotec GmbH, Bergisch Gladbach, Germany), with the addition of normal saline solution as necessary to obtain a heavy suspension. All manipulations were performed under sterile conditions and under laminar airflow. One hundred microliters of this suspension was inoculated on each of the following agar plates: (i) blood agar, i.e., Columbia-D agar base (bioMérieux, Marcy l'Etoile, France) with 5% sheep blood; (ii) chocolate agar, i.e., Columbia-D agar base (bioMérieux, Marcy l'Etoile, France) with 5% sheep blood (heated to lyse blood cells) supplemented with Vitox SR0090 growth factors (Oxoid-Thermo Fisher Scientific, Basingstoke, United Kingdom); or (iii) prereduced *Brucella* agar with 5% sheep blood, hemin, and vitamin K_1 (bioMérieux, Marcy l'Etoile, France). After inoculation, the plates were sealed with Parafilm laboratory film (Bemis Company, Inc., Oshkosh, WI) to avoid desiccation. The first 2 media were incubated at 35°C in a 5% CO_2 atmosphere for cultivation of aerobic and facultative organisms. The third plate was incubated at 35°C in an anaerobic atmosphere for the cultivation of anaerobic and facultative organisms. The remainder of the suspension was inoculated into thioglycolate broth medium CM0173 (Oxoid-Thermo Fisher Scientific, Basingstoke, United Kingdom) and incubated at 35°C for enrichment of aerobic, anaerobic, and facultative microorganisms. Quality control assessments showed the presence of adequate anaerobic conditions in the lower part of the broth. Each medium was inspected for signs of growth every day for a period of 14 days.

Statistical methods. Continuous variables are presented as medians and ranges and categorical variables as rates. Statistical significance was assessed using the chi-square test or Fisher's exact test for categorical variables and the Mann-Whitney *U* test (Kruskal-Wallis test) for continuous variables. All tests were performed using SPSS version 21 (SPSS Inc., Chicago, IL). *P* values of <0.05 were considered statistically significant. For graphical representation, Microsoft Excel 2008 (Microsoft Corp., Redmond, WA) was used.

TABLE 1 Spectrum of microorganisms in tissue biopsy specimens

Microorganism(s)	Infection (*n* [%])	Contamination (*n* [%])
Staphylococcus aureus	22 (37.9)	
Coagulase-negative staphylococci	10 (17.2)	8 (66.7)
Streptococcus spp.[a]	2 (3.4)	
Enterococcus spp.[b]	2 (3.4)	
Propionibacterium acnes	1 (1.7)	2 (16.7)
Gram-negative bacilli[c]	6 (10.3)	
Polymicrobial culture[d]	15 (25.9)	2 (16.7)
Total	58 (100)	12 (100)

[a] *Streptococcus* spp. included *Streptococcus* group *mitis* ($n = 1$) and *Streptococcus pyogenes* ($n = 1$).
[b] *Enterococcus* spp. included *Enterococcus faecalis* ($n = 1$) and *Enterococcus faecium* ($n = 1$).
[c] Gram-negative bacilli included *Escherichia coli* ($n = 3$), *Enterobacter cloacae* ($n = 2$), and *Pseudomonas aeruginosa* ($n = 1$).
[d] Polymicrobial cultures classified as infections included coagulase-negative staphylococci ($n = 11$), *S. aureus* ($n = 5$), *E. cloacae* ($n = 3$), *Bacillus* spp. ($n = 2$), *E. coli* ($n = 2$), *Streptococcus agalactiae* ($n = 1$), *E. faecalis* ($n = 1$), *Corynebacterium* sp. ($n = 1$), *Dermatobia hominis* ($n = 1$), *P. acnes* ($n = 1$), *Morganella morganii* ($n = 1$), *P. aeruginosa* ($n = 1$), and Gram-positive rods ($n = 1$). Polymicrobial cultures classified as contaminations included coagulase-negative staphylococci ($n = 1$), *E. faecalis* ($n = 1$), *E. coli* ($n = 1$), and Gram-negative bacilli ($n = 1$).

RESULTS

Study population. During the study period, 499 tissue biopsy specimens were collected from 117 cases of revision surgery, corresponding to a median number of 4.0 samples per case (range, 1 to 12 samples per case). In 70 cases (59.8%), a minimum of one sample was positive for microbiological growth, leaving 47 cases (40.2%) as sterile during the incubation period.

The study population consisted of 50 women (42.7%) and 67 men (57.3%), with a median age of 68.0 years (range, 14 to 94 years). The time between index surgery and revision surgery was <1 month in 31 cases (26.5%), 1 to 12 months in 32 cases (27.4%), and >12 months in 54 cases (46.2%). Orthopedic devices included 62 cases (53.0%) of joint prostheses and 55 cases (47.0%) of internal fixation devices. Localization of the devices varied from the hip in 51 cases (43.6%), the knee in 29 cases (24.8%), the lower extremity in 22 cases (18.8%), and the upper limb in 9 cases (7.7%) to the spine in 6 cases (5.1%). Articular devices were labeled according to the joint region involved. Histopathological analysis results were available for 85 cases (72.6%). Among the 70 cases with positive culture results, 58 cases (82.9%) were classified as infections and 12 cases (17.1%) as contaminations. The proportion of infections among the 117 cases of revision surgery was 49.6%, with 41.9% for joint replacement and 58.2% for internal fixation devices.

Microbiology. The majority of the isolated microorganisms were Gram-positive bacteria, mainly *Staphylococcus aureus* in 22 cases (31.4%) and coagulase-negative staphylococci in 18 cases (25.7%). *Streptococcus* spp. accounted for 2 cases (2.9%), *Enterococcus* spp. for 2 cases (2.9%), *P. acnes* for 3 cases (4.3%), Gram-negative bacteria for 6 cases (8.6%), and polymicrobial culture results for 17 cases (24.3%). The full spectrum of bacteria according to case classification is illustrated in Table 1. The two types of

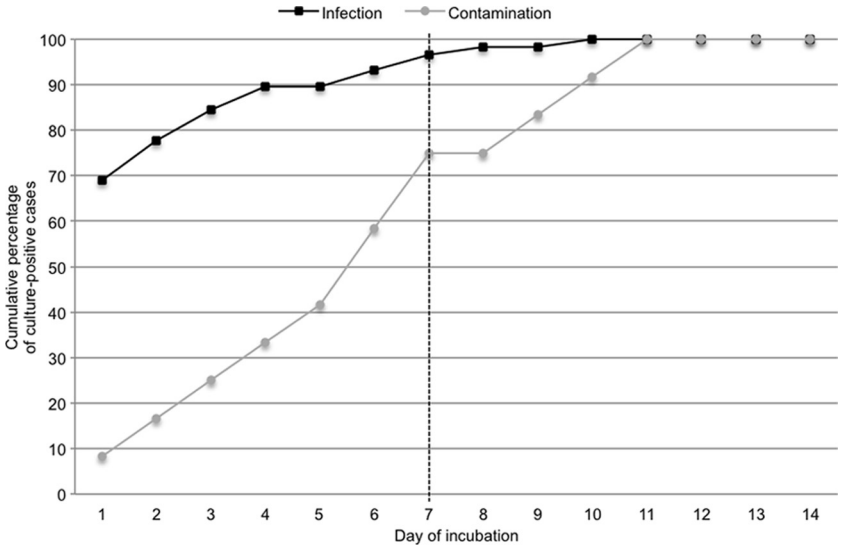

FIG 1 Time to tissue culture positivity for cases of infection versus contamination.

orthopedic devices showed similar spectra of microorganisms except for *S. aureus*, which was significantly more frequent for prostheses than for internal fixation devices (15 cases versus 7 cases; $P = 0.04$).

Diagnostic criteria. Of the 58 cases of infection, most were diagnosed by at least 2 diagnostic criteria, leaving only 9 cases diagnosed with a single criterion (the microbiological criterion in 3 cases and the clinical criterion in 6 cases). Discrepancy between the study's definition of infection and the treating medical team's diagnosis occurred in one case, which was treated as an infection based on one of 8 samples showing *P. acnes* on day 7. This case was classified as contamination according to our criteria.

Time to culture positivity. The median time to culture positivity for the 70 cases with positive results for tissue biopsy specimens was 1 day (range, 1 to 11 days). A total of 47 cases (67.1%) became positive within the first 2 days of incubation, 57 cases (81.4%) within 5 days, and 65 cases (92.9%) within 7 days.

The median time to positivity in cases classified as infections was 1 day (range, 1 to 10 days), compared to 6 days (range, 1 to 11 days) for cases considered contaminations ($P < 0.001$). A total of 52 cases (89.7%) of infections were diagnosed within 5 days of incubation and 56 cases (96.6%) within 7 days (Fig. 1). No infection was diagnosed beyond 10 days. Twenty-five percent of contaminants grew after 7 days, representing the majority (60%) of late-growing microorganisms. Because the absolute numbers of cases of infections or contaminations are also relevant, graphical representation of the results is shown in a histogram in Fig. 2.

Only 2 cases of infection were detected after 7 days of incubation, at day 8 and day 10 (Table 2). The first case was a late postoperative infection, which showed a large amount of *Corynebac-*

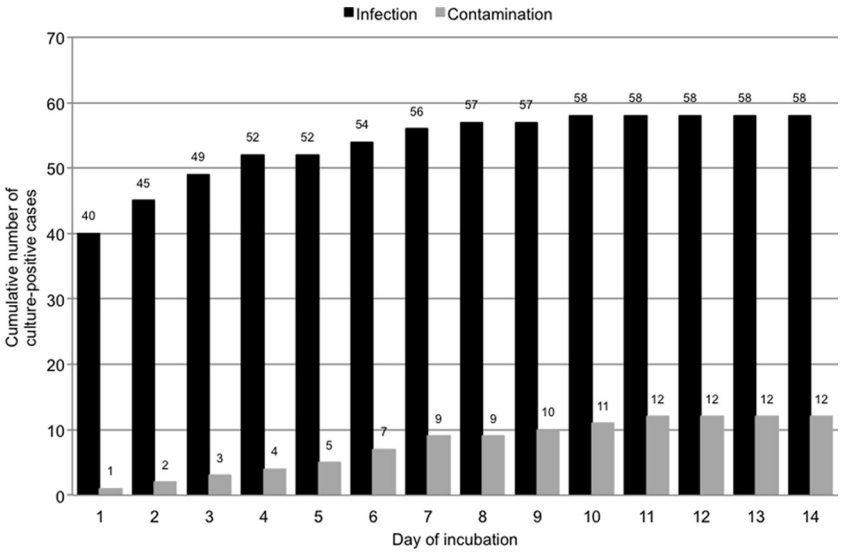

FIG 2 Absolute numbers of cases with positive tissue culture results at each day of incubation for infection versus contamination.

TABLE 2 Characteristics of 2 cases of infection with times to tissue culture positivity beyond 7 days of incubation

Case no.	Microorganism(s)	No. of culture-positive samples/no. tested	No. of PMN/HPF[a]	Type of implant	Clinical signs	Day of growth
1	*Corynebacterium* sp.	4/5	0	Hip prosthesis	Ongoing pain	4
	E. coli	1/5	0			8
2	Coagulase-negative staphylococci	1/3	>5	Knee prosthesis	Erythema, wound discharge	10

[a] Number of polymorphonuclear cells (PMN) per high-power field (HPF) (×400 magnification); >5 PMN/HPF is highly suggestive of infection.

terium sp. (4 of 5 positive tissue biopsy specimens) growing within 4 days. This was associated with an *Escherichia coli* infection (one of 5 positive tissue biopsy specimens) growing at day 8, and both bacteria were considered pathogens. The second case involved an early postoperative infection and demands special consideration. In this case, sampling occurred during antibiotic treatment, which had been initiated after a superficial wound swab showed *S. aureus* (not recorded in our database) in the presence of clinical signs of acute infection (erythema, edema, and wound discharge). Coagulase-negative staphylococci found to be growing in the tissue biopsy specimen after 10 days were recorded as the etiological agent, according to our study definition (histopathological and clinical criteria). However, considering the clinical picture, it is more probable that *S. aureus* was responsible for the infection. As a consequence, the late growth of coagulase-negative staphylococci is probably not relevant.

Our sample showed no significant difference in the median time to culture positivity ($P = 0.84$) with regard to the type of orthopedic device (joint replacement or internal fixation device).

DISCUSSION

Few studies have focused on the optimal incubation time for orthopedic surgical specimens. Most commonly, incubations of 5 to 7 days are used for ODAI (10, 11, 30–32). In recent studies (12, 13, 15), however, prolonging the incubation time for up to 14 days has been proposed. Schaefer et al. (13) addressed the infectious component in aseptic loosening and considered mostly elective surgery. Zappe et al. (15) and Butler-Wu et al. (12) specifically explored an extended culture protocol for *P. acnes*. In studies on alternative diagnostic procedures, such as 16S rRNA PCR or sonication, 14-day incubations have sometimes been reported, although without assessment of their benefits (33, 34). The aim of our study was to explore the relevance of 14-day tissue biopsy specimen culture incubations for the diagnosis of overall ODAI in the setting of general orthopedic and trauma surgery, where both acute and chronic infections are encountered.

We found that an incubation period of 7 days was sufficient to identify 56 of 58 cases (96.6%) of infection. The major difference between our data and those of Schaefer et al. (13) is the proportion of low-virulence microorganisms such as *Propionibacterium* spp., coryneform bacteria, and coagulase-negative staphylococci, which accounted for 80% of infections in their study population. These microorganisms are known to have slow growth rates. In our study, only 54% of the cases showed low-virulence microorganisms. In particular, *P. acnes* accounted for 4.3% of isolated bacteria, which corresponds to reports from other general orthopedic and trauma surgery departments (8, 35, 36).

The diagnosis of ODAI is a well-known challenge. In our study, we have tried to provide clear reproducible diagnostic criteria that are applicable to retrospective analysis, which has well-established limitations. For the microbiological criterion, our threshold of at least 3 positive samples with identical microorganisms could be viewed as stringent, in comparison with other studies in which 2 culture-positive specimens are considered sufficient for the diagnosis of infections (10, 37, 38). However, none of our contamination cases had more than one positive sample, meaning that our results would not have been different if we had adopted a lower threshold. The same is true for the histopathological criterion, as no contaminant showed an intermediate result of 1 to 5 PMN/HPF. Although the clinical diagnostic criterion is somewhat subjective, all of the cases classified as infections on this basis were quite evident; 10% of infections would have been missed without this strategy.

Overall, we identified a large proportion of infections (22, 39, 40). This can be explained by the inclusion of trauma cases, for which early revision surgery was indicated on the basis of a high preoperative suspicion of infection, whereas the systematic sampling of loose prostheses used in other studies obviously had lower yields (39, 40).

Most reports focus on hip and knee prostheses (1, 13, 24, 37). We believe that including both prostheses and internal fixation devices in our study makes sense because similar pathogens have been described in the two settings (41–43) and biofilm formation is common to all types of foreign-body infections (44–46). We found that slow-growing microorganisms such as coagulase negative-staphylococci and *P. acnes* were equally represented in the two groups.

In conclusion, the results of our study show that extension of culture incubation times beyond 7 days has a low yield in a general orthopedic and trauma setting, where virulent bacteria predominate and posttraumatic infections are frequent. However, based on the current literature, prolonging incubations to 14 days or using molecular techniques might be useful in particular situations in which the prevalence of slow-growing bacteria and anaerobes is higher.

REFERENCES

1. **Darouiche RO.** 2004. Treatment of infections associated with surgical implants. N. Engl. J. Med. **350:**1422–1429. http://dx.doi.org/10.1056/NEJMra035415.
2. **Kurtz SM, Lau E, Watson H, Schmier JK, Parvizi J.** 2012. Economic burden of periprosthetic joint infection in the United States. J. Arthroplasty **27:**61–65.e1. http://dx.doi.org/10.1016/j.arth.2012.02.022.
3. **Vanhegan IS, Malik AK, Jayakumar P, Ul Islam S, Haddad FS.** 2012. A financial analysis of revision hip arthroplasty: the economic burden in relation to the national tariff. J. Bone Joint Surg. Br. **94:**619–623. http://dx.doi.org/10.1302/0301-620X.94B5.27073.
4. **Zimmerli W, Moser C.** 2012. Pathogenesis and treatment concepts of orthopaedic biofilm infections. FEMS Immunol. Med. Microbiol. **65:**158–168. http://dx.doi.org/10.1111/j.1574-695X.2012.00938.x.
5. **Matthews PC, Berendt AR, McNally MA, Byren I.** 2009. Diagnosis and management of prosthetic joint infection. BMJ **338:**b1773. http://dx.doi.org/10.1136/bmj.b1773.

6. **Berbari E, Mabry T, Tsaras G, Spangehl M, Erwin PJ, Murad MH, Steckelberg J, Osmon D.** 2010. Inflammatory blood laboratory levels as markers of prosthetic joint infection: a systematic review and meta-analysis. J. Bone Joint Surg. Am. **92:**2102–2109. http://dx.doi.org/10.2106/JBJS.I.01199.
7. **Parvizi J, Zmistowski B, Berbari EF, Bauer TW, Springer BD, Della Valle CJ.** 2011. New definition for periprosthetic joint infection from the workgroup of the Musculoskeletal Infection Society. Clin. Orthop. Relat. Res. **469:**2992–2994. http://dx.doi.org/10.1007/s11999-011-2102-9.
8. **Zimmerli W, Trampuz A, Ochsner PE.** 2004. Prosthetic-joint infections. N. Engl. J. Med. **351:**1645–1654. http://dx.doi.org/10.1056/NEJMra040181.
9. **Larsen LH, Lange J, Xu Y, Schonheyder HC.** 2012. Optimizing culture methods for diagnosis of prosthetic joint infections: a summary of modifications and improvements reported since 1995. J. Med. Microbiol. **61:**309–316. http://dx.doi.org/10.1099/jmm.0.035303-0.
10. **Piper KE, Jacobson MJ, Cofield RH, Sperling JW, Sanchez-Sotelo J, Osmon DR, McDowell A, Patrick S, Steckelberg JM, Mandrekar JN, Fernandez Sampedro M, Patel R.** 2009. Microbiologic diagnosis of prosthetic shoulder infection by use of implant sonication. J. Clin. Microbiol. **47:**1878–1884. http://dx.doi.org/10.1128/JCM.01686-08.
11. **Font-Vizcarra L, Garcia S, Martinez-Pastor JC, Sierra JM, Soriano A.** 2010. Blood culture flasks for culturing synovial fluid in prosthetic joint infections. Clin. Orthop. Relat. Res. **468:**2238–2243. http://dx.doi.org/10.1007/s11999-010-1254-3.
12. **Butler-Wu SM, Burns EM, Pottinger PS, Magaret AS, Rakeman JL, Matsen FA, III, Cookson BT.** 2011. Optimization of periprosthetic culture for diagnosis of *Propionibacterium acnes* prosthetic joint infection. J. Clin. Microbiol. **49:**2490–2495. http://dx.doi.org/10.1128/JCM.00450-11.
13. **Schaefer P, Fink B, Sandow D, Margull A, Berger I, Frommelt L.** 2008. Prolonged bacterial culture to identify late periprosthetic joint infection: a promising strategy. Clin. Infect. Dis. **47:**1403–1409. http://dx.doi.org/10.1086/592973.
14. **Gollwitzer H, Diehl P, Gerdesmeyer L, Mittelmeier W.** 2006. Diagnostic strategies in cases of suspected periprosthetic infection of the knee: a review of the literature and current recommendations. Orthopade **35:**904–916. (In German.) http://dx.doi.org/10.1007/s00132-006-0977-z.
15. **Zappe B, Graf S, Ochsner PE, Zimmerli W, Sendi P.** 2008. *Propionibacterium* spp in prosthetic joint infections: a diagnostic challenge. Arch. Orthop. Trauma Surg. **128:**1039–1046. http://dx.doi.org/10.1007/s00402-007-0454-0.
16. **Costerton JW, Cheng KJ, Geesey GG, Ladd TI, Nickel JC, Dasgupta M, Marrie TJ.** 1987. Bacterial biofilms in nature and disease. Annu. Rev. Microbiol. **41:**435–464.
17. **Ceri H, Olson ME, Stremick C, Read RR, Morck D, Buret A.** 1999. The Calgary Biofilm Device: new technology for rapid determination of antibiotic susceptibilities of bacterial biofilms. J. Clin. Microbiol. **37:**1771–1776.
18. **Anderl JN, Zahller J, Roe F, Stewart PS.** 2003. Role of nutrient limitation and stationary-phase existence in *Klebsiella pneumoniae* biofilm resistance to ampicillin and ciprofloxacin. Antimicrob. Agents Chemother. **47:**1251–1256. http://dx.doi.org/10.1128/AAC.47.4.1251-1256.2003.
19. **Chuard C, Lucet JC, Rohner P, Herrmann M, Auckenthaler R, Waldvogel FA, Lew DP.** 1991. Resistance of *Staphylococcus aureus* recovered from infected foreign body *in vivo* to killing by antimicrobials. J. Infect. Dis. **163:**1369–1373.
20. **Atkins BL, Athanasou N, Deeks JJ, Crook DWM, Simpson H, Peto TEA, McLardy-Smith P, Berendt AR, OSIRIS Collaborative Study Group.** 1998. Prospective evaluation of criteria for microbiological diagnosis of prosthetic-joint infection at revision arthroplasty. J. Clin. Microbiol. **36:**2932–2939.
21. **Pandey R, Drakoulakis E, Athanasou NA.** 1999. An assessment of the histological criteria used to diagnose infection in hip revision arthroplasty tissues. J. Clin. Pathol. **52:**118–123.
22. **Lonner JH, Desai P, Dicesare PE, Steiner G, Zuckerman JD.** 1996. The reliability of analysis of intraoperative frozen sections for identifying active infection during revision hip or knee arthroplasty. J. Bone Joint Surg. Am. **78:**1553–1558.
23. **Mirra JM, Amstutz HC, Matos M, Gold R.** 1976. The pathology of the joint tissues and its clinical relevance in prosthesis failure. Clin. Orthop. Relat. Res. (**117**):221–240.
24. **Widmer AF.** 2001. New developments in diagnosis and treatment of infection in orthopedic implants. Clin. Infect. Dis. **33**(Suppl 2)**:**S94–S106. http://dx.doi.org/10.1086/321863.
25. **Senneville E, Savage C, Nallet I, Yazdanpanah Y, Giraud F, Migaud H, Dubreuil L, Courcol R, Mouton Y.** 2006. Improved aero-anaerobe recovery from infected prosthetic joint samples taken from 72 patients and collected intraoperatively in Rosenow's broth. Acta Orthop. **77:**120–124. http://dx.doi.org/10.1080/17453670610045795.
26. **Zuluaga AF, Galvis W, Saldarriaga JG, Agudelo M, Salazar BE, Vesga O.** 2006. Etiologic diagnosis of chronic osteomyelitis: a prospective study. Arch. Intern. Med. **166:**95–100. http://dx.doi.org/10.1001/archinte.166.1.95.
27. **Aggarwal VK, Higuera C, Deirmengian G, Parvizi J, Austin MS.** 2013. Swab cultures are not as effective as tissue cultures for diagnosis of periprosthetic joint infection. Clin. Orthop. Relat. Res. **471:**3196–3203. http://dx.doi.org/10.1007/s11999-013-2974-y.
28. **Bori G, Munoz-Mahamud E, Garcia S, Mallofre C, Gallart X.** 2011. Interface membrane is the best sample for histological study to diagnose prosthetic joint infection. Mod. Pathol. **24:**579–584. http://dx.doi.org/10.1038/modpathol.2010.219.
29. **Bjerkan G, Witsoe E, Nor A, Viset T, Loeseth K, Lydersen S.** 2012. A comprehensive microbiological evaluation of fifty-four patients undergoing revision surgery due to prosthetic joint loosening. J. Med. Microbiol. **61:**572–581. http://dx.doi.org/10.1099/jmm.0.036087-0.
30. **Virolainen P, Lahteenmaki H, Hiltunen A, Sipola E, Meurman O, Nelimarkka O.** 2002. The reliability of diagnosis of infection during revision arthroplasties. Scand. J. Surg. **91:**178–181.
31. **Trampuz A, Piper KE, Jacobson MJ, Hanssen AD, Unni KK, Osmon DR, Mandrekar JN, Cockerill FR, Steckelberg JM, Greenleaf JF, Patel R.** 2007. Sonication of removed hip and knee prostheses for diagnosis of infection. N. Engl. J. Med. **357:**654–663. http://dx.doi.org/10.1056/NEJMoa061588.
32. **Dempsey KE, Riggio MP, Lennon A, Hannah VE, Ramage G, Allan D, Bagg J.** 2007. Identification of bacteria on the surface of clinically infected and non-infected prosthetic hip joints removed during revision arthroplasties by 16S rRNA gene sequencing and by microbiological culture. Arthritis Res. Ther. **9:**R46. http://dx.doi.org/10.1186/ar2201.
33. **Ince A, Rupp J, Frommelt L, Katzer A, Gille J, Lohr JF.** 2004. Is "aseptic" loosening of the prosthetic cup after total hip replacement due to nonculturable bacterial pathogens in patients with low-grade infection? Clin. Infect. Dis. **39:**1599–1603. http://dx.doi.org/10.1086/425303.
34. **Sendi P, Frei R, Maurer TB, Trampuz A, Zimmerli W, Graber P.** 2010. *Escherichia coli* variants in periprosthetic joint infection: diagnostic challenges with sessile bacteria and sonication. J. Clin. Microbiol. **48:**1720–1725. http://dx.doi.org/10.1128/JCM.01562-09.
35. **Moran E, Masters S, Berendt AR, McLardy-Smith P, Byren I, Atkins BL.** 2007. Guiding empirical antibiotic therapy in orthopaedics: the microbiology of prosthetic joint infection managed by debridement, irrigation and prosthesis retention. J. Infect. **55:**1–7. http://dx.doi.org/10.1016/j.jinf.2007.01.007.
36. **Fulkerson E, Valle CJ, Wise B, Walsh M, Preston C, Di Cesare PE.** 2006. Antibiotic susceptibility of bacteria infecting total joint arthroplasty sites. J. Bone Joint Surg. Am. **88:**1231–1237. http://dx.doi.org/10.2106/JBJS.E.00004.
37. **Osmon DR, Berbari EF, Berendt AR, Lew D, Zimmerli W, Steckelberg JM, Rao N, Hanssen A, Wilson WR.** 2013. Diagnosis and management of prosthetic joint infection: clinical practice guidelines by the Infectious Diseases Society of America. Clin. Infect. Dis. **56:**e1–e25. http://dx.doi.org/10.1093/cid/cis803.
38. **Workgroup Convened by the Musculoskeletal Infection Society.** 2011. New definition for periprosthetic joint infection. J. Arthroplasty **26:**1136–1138. http://dx.doi.org/10.1016/j.arth.2011.09.026.
39. **Zimmerli W, Widmer AF, Blatter M, Frei R, Ochsner PE.** 1998. Role of rifampin for treatment of orthopedic implant-related staphylococcal infections: a randomized controlled trial. JAMA **279:**1537–1541.
40. **Padgett DE, Silverman A, Sachjowicz F, Simpson RB, Rosenberg AG, Galante JO.** 1995. Efficacy of intraoperative cultures obtained during revision total hip arthroplasty. J. Arthroplasty **10:**420–426.
41. **Prasarn ML, Ouellette EA, Miller DR.** 2010. Infected nonunions of diaphyseal fractures of the forearm. Arch. Orthop. Trauma Surg. **130:**867–873. http://dx.doi.org/10.1007/s00402-009-1016-4.
42. **Trampuz A, Widmer AF.** 2006. Infections associated with orthopedic implants. Curr. Opin. Infect. Dis. **19:**349–356.
43. **Esteban J, Sandoval E, Cordero-Ampuero J, Molina-Manso D, Ortiz-Perez A, Fernandez-Roblas R, Gomez-Barrena E.** 2012. Sonication of intramed-

ullary nails: clinically-related infection and contamination. Open Orthop. J. **6:**255–260. http://dx.doi.org/10.2174/1874325001206010255.

44. **Stoodley P, Nistico L, Johnson S, Lasko LA, Baratz M, Gahlot V, Ehrlich GD, Kathju S.** 2008. Direct demonstration of viable *Staphylococcus aureus* biofilms in an infected total joint arthroplasty: a case report. J. Bone Joint Surg. Am. **90:**1751–1758. http://dx.doi.org/10.2106/JBJS.G.00838.
45. **Palmer M, Costerton W, Sewecke J, Altman D.** 2011. Molecular techniques to detect biofilm bacteria in long bone nonunion: a case report. Clin. Orthop. Relat. Res. **469:**3037–3042. http://dx.doi.org/10.1007/s11999-011-1843-9.
46. **Moriarty TF, Schlegel U, Perren S, Richards RG.** 2010. Infection in fracture fixation: can we influence infection rates through implant design? J. Mater. Sci. Mater. Med. **21:**1031–1035. http://dx.doi.org/10.1007/s10856-009-3907-x.